

CLAIMS

What is claimed as the invention is:

1. A composition comprising proliferating primate pluripotent stem (pPS) cells, which is essentially free of feeder cells.
2. The composition of claim 1, further comprising a conditioned medium produced by collecting medium from a culture of feeder cells.
3. The composition of claim 1, further comprising extracellular matrix components (such as Matrigel®, laminin, or collagen).
4. A method for culturing primate pluripotent stem (pPS) cells, comprising culturing pPS cells in a growth environment essentially free of feeder cells but containing conditioned medium produced by collecting medium from a culture of feeder cells.
5. A method for producing a conditioned medium suitable for culturing primate pluripotent stem (pPS) cells in a growth environment essentially free of feeder cells, comprising:
  - a) conditioning medium by culturing cells in the medium, wherein the cells are a euploid cell line that can proliferate in culture for at least 60 days; and
  - b) harvesting the conditioned medium.
6. Conditioned medium to support culturing primate pluripotent stem (pPS) cells in a growth environment essentially free of feeder cells, produced according to the method of claim 5.
7. The composition of claim 2, wherein the cell line used to produce the conditioned medium has one or more of the following properties:
  - i) it is euploid;
  - ii) it is an immortalized mouse cell line;
  - iii) it is a human cell line;
  - iv) it is a fibroblast cell line; or
  - v) it can proliferate in culture for at least 60 days.
8. A human cell line obtained by differentiating a culture of human embryonic stem (hES) cells into a population of differentiated cells that comprises fibroblast-like cells, and then selecting fibroblast-like cells from the culture; wherein conditioned medium produced by harvesting medium from a culture of the fibroblast-like cells supports growth of pPS cells in a culture environment essentially free of feeder cells.
9. The composition of claim 2, wherein the cell line used to produce the conditioned medium has been genetically altered to express telomerase reverse transcriptase (TERT) at an elevated level.

10. A method of producing a differentiated cell population, comprising causing or permitting cells of a composition according to claim 1 to differentiate.
11. A method for producing differentiated cells from a donor culture of undifferentiated primate pluripotent stem (pPS) cells, comprising:
  - a) preparing a suspension of cells from the undifferentiated donor culture;
  - b) replating and culturing the suspended cells on a solid surface so that they differentiate without forming embryoid bodies; and
  - c) harvesting differentiated cells from the solid surface.
12. A method for producing differentiated cells from a donor culture of primate pluripotent stem (pPS) cells, comprising:
  - a) providing a culture of primate pluripotent stem (pPS) cells that is essentially free of feeder cells;
  - b) changing the medium in which the cells are cultured; and
  - c) harvesting differentiated cells after culturing for a time in the changed medium.
13. The method of claim 11, wherein the donor culture of pPS cells is a culture essentially free of feeder cells, according to any of claims .
14. The method of claim 11, having at least one of the following features:
  - i) the solid surface bears a poly-cation (such as poly-lysine or poly-ornithine);
  - ii) differentiation is promoted by withdrawing serum, serum replacement, or a factor that inhibits differentiation from medium in which the cells are cultured after replating; or
  - iii) differentiation is promoted by adding a factor (such as Brain Derived Neurotrophic Factor, BDNF; or Neurtrophin-3, NT-3) that promotes differentiation in medium in which the cells are cultured after replating.
15. A differentiated cell population produced by the method of claim 10.
16. A method of screening a compound for cellular toxicity or modulation, comprising contacting a differentiated cell according to claim 15 with the compound, determining any phenotypic or metabolic changes in the cell that result from contact with the compound, and correlating the change with cellular toxicity or modulation.
17. A method for producing a polynucleotide comprising a nucleotide sequence contained in an mRNA that is expressed at a different level in committed or differentiated cells compared with undifferentiated primate pluripotent stem (pPS) cells, the method comprising:
  - a) determining the level of expression of a plurality of mRNAs in committed or differentiated cells, in comparison to the level of expression of the same mRNAs in undifferentiated pPS cells;
  - b) identifying an mRNA expressed at a different level in the committed or differentiated cells, relative to the undifferentiated pPS cells; and
  - c) preparing a polynucleotide comprising a nucleotide sequence of at least 30 consecutive nucleotides contained in the identified mRNA.

18. A method of producing genetically altered primate pluripotent stem (pPS) cells, comprising:
- a) providing a composition of pPS cells essentially free of feeder cells according to claim 1;
  - b) transferring a polynucleotide into pPS cells in the composition; and then optionally
  - c) preferentially selecting cells that have been genetically altered with the polynucleotide.
19. A method of producing genetically altered primate pluripotent stem (pPS) cells, comprising:
- a) providing a composition of pPS cells on a layer of feeder cells that are drug-resistant;
  - b) transferring a polynucleotide into pPS cells in the composition; and
  - c) selecting genetically altered cells in the composition using the drug to which the feeder cells are resistant.
20. The method of claim 18, wherein the polynucleotide comprises a protein encoding region operably linked to a promoter that promotes transcription of the encoding region in an undifferentiated pPS cell.
21. A population of primate pluripotent stem (pPS) cells, in which at least 25% of the undifferentiated pPS cells have been stably transfected with a polynucleotide, or are the progeny of such cells that have inherited the polynucleotide.
22. A population of genetically altered differentiated cells, obtained by differentiating the cells of claim 21.
23. A method of producing an mRNA preparation or a cDNA library from primate pluripotent stem (pPS) cells before or after differentiation, comprising providing a culture of undifferentiated pPS cells essentially free of feeder cells, optionally permitting the pPS cells to differentiate, and isolating mRNA from the undifferentiated or differentiated cells.
24. The method of claim 23, comprising isolating mRNA from pPS cells in a culture essentially free of feeder cells, and recombining cDNA copies of the mRNA into a cloning vector, wherein the cDNA copies are operatively linked to a transcriptional regulatory control element (such as the PGK promoter) that promotes transcription of the cDNA in undifferentiated pPS cells.
25. The method of claim 23, which is a method for producing a cDNA subtraction library enriched for transcripts differently expressed in a first cell population compared with a second cell population, comprising incubating together preparations of mRNA (or cDNA copies thereof) obtained from the first and second cell populations under conditions that permit polynucleotides present in both preparations to cross-hybridize; and then recombining polynucleotides that have not cross-hybridized into a cloning vector.
26. A cDNA library produced according to the method of claim 23.
27. A cDNA library of at least 1,000 genes expressed at the mRNA level in either undifferentiated pPS cells, or cells differentiated from pPS cells, wherein the library is essentially free of cDNA of other vertebrates.

28. The cDNA library of claim 26, wherein at least 30% of cDNA segments in the library comprise the entire encoding region of the corresponding mRNA.
29. A method for producing a polynucleotide containing a sequence of an mRNA expressed in undifferentiated or differentiated pPS cells, comprising determining nucleotide sequence from an mRNA or cDNA obtained according to claim 23, and manufacturing a polynucleotide containing the determined sequence.
30. A method for producing an amino acid containing a sequence of a polypeptide expressed in undifferentiated or differentiated pPS cells, comprising determining amino acid sequence from a protein encoding region of an mRNA or cDNA obtained according to claim 23, and manufacturing a protein containing the determined sequence.
31. A method for producing an antibody specific for a polypeptide expressed in undifferentiated or differentiated pPS cells, comprising determining amino acid sequence from a protein encoding region of an mRNA or cDNA obtained according to claim 23, and immunizing an animal or contacting an immunocompetent cell or particle with a protein containing the determined sequence.
32. The composition of claim 1, wherein the pPS cells are human embryonic stem (hES) cells.
33. A method of establishing a line of human embryonic stem cells, comprising:  
a) isolating cells from the inner cell mass of a human blastocyst;  
b) forming colonies comprising undifferentiated cells from the isolated blastocyst cells;  
c) passaging the colonies into a culture environment that is essentially free of feeder cells; and  
d) culturing the colonies in the culture environment, thereby establishing a line of human embryonic stem cells;  
wherein the embryonic stem cell line can be caused to proliferate in culture for at least 64 days without differentiation, while maintaining a normal karyotype and the potential to differentiate to cells of endoderm, mesoderm, and ectoderm tissues.
34. The method of claim 33, wherein at least ~80% of the cells in the culture environment express SSEA-4, telomerase reverse transcriptase (TERT), and OCT-4.
35. A method for producing a population of differentiated cells, comprising:  
a) obtaining a line of embryonic stem cells that have been established in a culture environment that is essentially free of feeder cells; and  
b) causing cells in the culture to differentiate into the population of differentiated cells.
36. A human embryonic stem cell line, established according to the method of claim 33.